resulting in increased GSK3β activity. IGF-1 (100nM) resulted in increased phosphorylation of Akt. Our results demonstrated that a reduction in phospho-Akt and subsequent increase in GSK3β activity was associated with ER stress-mediated apoptosis. Activation of Akt by IGF-1 resulted in amelioration of ER stress-induced apoptosis. These results strongly suggest that the PI-3-kinase pathway appears to play an important role in ER stress-mediated apoptosis in β-cells. Akt and GSK3β may be possible targets for pharmacological intervention to promote β-cell survival. Drugs that inhibit GSK3β may have therapeutic potential for the treatment of diabetes. **ADA Funded Research**

1545-P

IL-1β Treatment Is Associated with Detrimental Nuclear Changes in Insulin Secreting RINm5F Cells

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Proinflammatory cytokines [e.g., IL-1β] have been shown to induce metabolic dysfunction and demise of the islet β cell. The degradation of nuclear lamin-B represents one of the proposed events in the apoptosis of the β cell induced by IL. Hyperphosphorylation by PKCδ appears to render lamin-B susceptible to degradation by caspase 6. The present study was aimed at deciphering changes in the nucleus of the β cell following IL-challenge. IL-1β-treatment [600 pM, 48 hrs] of RINm5F cells resulted in significant degradation of DNA as evidenced by increased DNA laddering and association of lamin-B with DNA fragments [DAPI staining; confocal microscopy]. The nuclear membrane, soluble and Triton X-100 insoluble [i.e., matrix] fractions were isolated from control and IL-treated cells by differential centrifugation. Western blot analysis suggested significant decrease in the content of lamin-B in the nuclear membrane with a concomitant increase in the matrix fraction in cells treated with IL-1β; observations compatible with our confocal data demonstrating similar pattern of distribution of lamin-B in IL-treated cells. A significant degree of PKCδ-dependent, rottlerin-sensitive phophorylation of lamin-B was also demonstrable in the nuclear fraction of the islet β cell. Further, IL-treatment significantly increased colocalization of lamin-B with caspase 6, a lamin-B-degrading protease. Together, our data provide the first evidence to indicate that IL-treatment results in significant alterations in the nuclear fraction of the β cell, specifically favoring conditions for nuclear lamin degradation leading to the demise of the effete β cell. **ADA Funded Research**

1546-P

Small Interfering RNA Inhibition of Cytokine-Induced Fas Expression on βTC-3 Cells

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Fas-mediated apoptosis may represent one mechanism underlying the destruction of islet cells following islet cell transplantation. Therefore, efficient suppression of Fas expression on islet cells through RNA interference may provide a powerful approach leading to the protection of β cells from immunological destruction and functional loss. Increased research interest has recently been directed at small interfering RNA (siRNA) molecules, given their potential to provide specific and robust gene silencing. The current study tested whether siRNA were capable of inhibiting Fas production and subsequent expression on mouse insulinoma βTC-3 cells. Three candidate siRNAs (21 nucleotides) were synthesized based upon the sequence and secondary structure of the murine Fas mRNA. Successful transfection of βTC-3 cells by Cy3-labeled Fas siRNAs was achieved with lipofectamine 2000 and confirmed by fluorescent microscopy. siRNAs were distributed in both cytoplasm and nuclei, with higher transfection efficiency observed in dividing cells than in resting cells. Treatment of βTC-3 cells for 16h with IL-1β and INF-γ increased the percentage of Fas-expressing cells from 25.1% to 50.5%. Interestingly, pretreatment (24h) of these cells with one of the three Fas siRNAs (10 nM) markedly reduced Fas-expressing cells to 28.1%, a percentage similar to that of untreated cells (P=NS). These results provide strong support to notion that RNA interference, through the use of siRNA, may represent a highly effecient means for gene inhibition in pancreatic β cells and provide a novel therapeutic tool for enhancing the effeciency of islet cell transplantation.

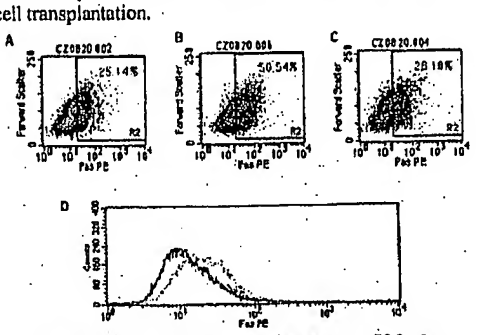

Fig. 1: siRNA inhibition of cytokine-induced Fas expression on βTC-3 cells. Flow cytometric profiles of βTC-3 cells of three different groups: untreated (A), cytokine-treated (B) and cytokine/Fas siRNA #3-treated (C). These profiles were overlaid in the panel D (untreated: black, cytokine-treated: blue, cytokine/Fas siRNA #3-treated: red).

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1547-P

Flow-Cytometric Analysis of Pancreatic Islet-Derived Progenitor Cells

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Islet-transplantation is now considered one of the best therapeutic options for the treatment of diabetes. However, the paucity of donor pancreases limits this approach and calls for investigation addressing alternate therapies such as a stem cell based therapy. Recent studies indicate the presence of a newly identified cell population within embryonic and adult pancreatic islets and ducts that expresses the neural-stem-cell protein Nestin. Confluent cultures of *N*estin-positive *I*slet-derived *P*rogenitor cell (NIPs) form Islet-Like Clusters (ILC's) and can be differentiated ex vivo into pancreatic endocrine, exocrine and hepatic phenotypes (Diabetes 50: 521). Our current research seeks to further characterize surface markers on NIPs by FACS analysis that will enable us to isolate and enrich specific populations of cells within NIP cultures that may differentiate into endocrine lineages. Accordingly, both mouse and human NIP cultures (^{3} passage 04) were immunoassayed with antibodies against several hematopoietic and non-hematopoietic antigens including CD34, 45, 133, 117, Sca-1 (mouse), 135/Flk-1 and CD 29, 49f, MHC.I & II, c-met, Glut-2, CK-19, GLP-1 receptor, SSEA (1-4), respectively. Although there are subtle differences between surface expression of mouse and human NIPs, they are largely negative for hematopoietic antigens. Notably, > 95% of NIPs are positive for integrins (CD29 and 49f). Immunoreactivity to GLP-1 receptors as well as SSEA-4, an embryonic stem cell antigen is abundant. Varying sub-populations of cells expressing Glut-2, c-Met and CK-19 were also detected indicating heterogeneity of NIP cultures.

FACS analysis of NIPs transfected with a retroviral-GFP construct gave rise to morphologically distinct cells that now express CD105, a mesenchymal stem cell marker. We also identified nestin-positive cells in mouse islet cultures, which appear to be endothelial in origin in that they express Flk-1. However, the latter cells bear no similarity with cloned NIPs in that they do not form ILC's and may arise from contamination of islet cultures.

1548-P

Glucagon like Peptide-1 Promotes Differentiation of Insulin-Producing Cells from Mouse Embryonic Stem Cells

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Embryonic stem (ES) cells have the ability to proliferate while remaining in an undifferentiated state, thus providing a potentially unlimited source of cells for medical/scientific purposes. ES cells are pluripotent, developing into all cell lineages, ecto-, meso- and endoderm. Endodermal cells represent a source of pancreatic stem cells that can develop into insulin-producing cells. Glucagon-like peptide-1 (GLP-1), a gut hormone, has been demonstrated to induce the differentiation of b cells from ductal progenitor cells in both adult and fetal pancreas. The aim of this study was to convert mouse ES cells into insulin-producing cells and improve the efficiency of such conversion by adding GLP-1. Mouse ES cells were cultured in 5 stages by a modification of a method previously described (*Science 2001; 292: 1389-94*) - stage 1: expansion of undifferentiated mES cells with leukemia inhibitory factor; stage 2: generation of embryoid bodies; stage 3: selection of progenitor cells in a defined medium supplemented with ITSFn (insulin, transferring, selenium and fibronectin); stage 4: expansion of progenitor cells by addition of basic fibroblast growth factor (bFGF); stage 5 differentiation into insulin-producing cells by removing bFGF and adding nicotinamide or nicotimide plus GLP-1. Cell development was characterized at each stage by gene expression. Oct-4, a marker of undifferentiated ES cells, was downregulated at the late stages of development and was absent at stage 5. Insulin and glucokinase, markers of β cells, were present at stage 5. Glucose transporter 2 (GLUT2) was present from stage 3; levels were enhanced in stage 5, especially in the presence of GLP-1 100 nM. Addition of GLP-1 in addition to nicotinamide 10 mM at stage 5 resulted in a 50% increase in insulin content compared with nicotinamide alone. Insulin secretion was enhanced 6-fold when exposed to 20 mM glucose. We conclude that it is possible to convert mouse ES cells into glucose-responsive insulin producing cells by varying the culture conditions. GLP-1 enhances the differentiation.

For author duality of interest information, see page A675.